CHROMOSOM V BUNĚČNÉM CYKLU

Cell cycle

prophase
metaphase
anaphase
telophase
CHROMOSOME CONDENSATION

MITOTIC CHROMOSOME

Chromosome condensation

• condensin complex - a key player
• required for proper chromosome condensation and segregation
• ATP dependent positive supercoiling in closed circular DNA has led to the suggestion that chromosome condensation results from the generation of a global positive writhe.
• at present, however, the mechanism by which condensin acts on the chromatin template is unclear.
Mitotic chromosome condensation requires phosphorylation of histone H3 tail at serine 10

- Mitotic chromosome condensation requires phosphorylation of histone H3 tail at serine 10

- Histone H3 exhibits site-specific phosphorylation at serine 10 during mitosis

- This histone H3 modifications is coupled tightly to the initiation of chromosome condensation but is not required for the maintenance of chromosome compaction

- Second mitotic-specific phosphorylation site at serine 28 of histone H3 was identified recently (its role unclear)

- Two candidate kinases: NIMA kinase and the Ipl1/aurora

Mechanical properties of chromatin/chromosomes

Based on force–distance measurements of individual chromatin fibers.

F<sub>ext</sub> is the extension force applied
Mechanical properties of chromosomes

• micromanipulations: mitotic chromosomes are highly extensible objects
• reversible stretching up to 10 times their original length
• ‘chromosomes’ assembled in Xenopus extracts: stretched up to 100 times their original length
• the relationship between the measured longitudinal deformability and the bending rigidity was remarkable: the rigidity was 2000 times less than that calculated from the experimental force–extension curve

• this best fits a model in which chromosomes are constructed of thin elastically-deformable rigid axes surrounded by a soft chromatin envelope
• quite different from the widely discussed scaffold loop and hierarchical helical folding models of chromosome structure.
• the elastic properties of the axes can be approximated by titin-like molecules
• genetic evidence - mutations in a *Drosophila* titin homolog disrupt chromosome condensation and mitosis
Major proteins of mitotic chromosomes

- histons
- DNA topoisomerase II
- condensin complex

A model for the architecture of the condensin complex

[Diagram showing the architecture of the condensin complex with labels for Cnd1 (XCAP-D2, Eg7, Ycs4, Loc7), Cnd2 (XCAP-H, Barren, Brn1), Cnd3 (XCAP-G, Ycg1, Ycs5), SMC2 (XCAP-E, Cut14), and SMC4 (XCAP-C, Gluon, Cut3).]
Condensin architecture and interaction with DNA: regulatory non-SMC subunits bind to the head of SMC heterodimer

AFM

Drosophila gluon gene

A role of condensin specifically in compacting chromosome loops is proposed, while the organisation of the chromosome axis is performed by different proteins, maybe involving cohesin.
MITOTIC CHROMOSOME

Levels of chromatin condensation

factor ~ 50,000x
Homo sapiens

Mitotic chromosomes in scanning electron microscope
G - banding

G-banding of human chromosomes
Chimpanzee karyotype – C banding
Harlequin chromosomes: sister chromatid exchanges

Human chromosome painting
FIDELITY SORTING OF CHROMOSOMES

The problem

• replicated chromosomes (sister chromatids) are segregated into newly-forming daughter cells
• assurance that they inherit a genome identical to the parental cell
• unusual sorting process because the two sister chromatids are identical macromolecules that must be moved to different places
• alternatively, a passive mechanism like diffusion can distribute identical molecules to different places, but the fidelity of this mechanism is limited by its stochastic nature
The solution – three specialised structures

• **sister chromatid cohesion**, cross-links between sister chromatids that form during DNA replication and persist until the onset of segregation in anaphase.

• **the centromere**, a specialized locus of the chromatid that organizes the assembly of a microtubule-binding complex called the **kinetochore**

• **the spindle**, a complex microtubule machine
Sister chromatid cohesion

• along the entire length of the sisters
• cohesion proximal to the centromeres is thought to orientate sister kinetochores so that they tend to attach to the microtubules emanating from opposite spindle poles
• after kinetochore–microtubule attachment, each sister chromatid experiences microtubule-dependent poleward forces
• these opposing forces are not powerful enough to overcome cohesion and initiate segregation of the sister chromatids, but instead they generate tension on them
• tension on all pairs of sister chromatids is thought to be a signal for their successful bipolar attachment and for the cell to inactivate cohesion and initiate anaphase
Players in chromatid cohesion

- **cohesin complex**: holds sisters together
- **separin**: sister-separating protein, destroys cohesion
- Separins are bound by **inhibitory proteins**
- Proteolysis of inhibitors at the metaphase–anaphase transition is mediated by the **Anaphase-Promoting Complex** and its activator protein **CDC20 (APCCDC20)**
- When chromosomes are **misaligned**, a **surveillance mechanism** (checkpoint) blocks sister separation by inhibiting APC CDC20.
- Defects in this apparatus are implicated in causing **aneuploidy** in human cells.

Candidates for the ‘glue’ (condensins)

Two obvious criteria for the ‘glue’ molecules that directly hold sister chromatids together:

- Necessary for the **maintenance** of sister chromatid cohesion (i.e., their inactivation after cohesion is established should cause sister chromatid dissociation)
- Should be present on sister chromatids at least **from S to M phase**
Candidates for the ‘glue’

• budding yeast: protein Mcd1/Scc, in complex with three other cohesion factors, Scc3, Smc1 and Smc3

• this cohesin complex conserved from yeast to vertebrates

• Smc1 and Smc3 are members of the SMC (structural maintenance of chromosomes) family of proteins

• SMC members - implicated in recombination, dosage compensation, transcription, silencing and chromosome condensation

SMC PROTEIN STRUCTURE

[Diagram showing the SMC protein structure with Walker A and Walker B domains, hinge, N-coiled-coil, C-coiled-coil, folding, dimerisation, and labels for Smc1 and Smc3]
The ring model of cohesin structure:
• the heterodimer **Smc1/Smc3** encircles sister chromatids
• this ring is closed by **Scc1** that binds the ATPase domains of both Smc1 and Smc3 through its C-terminal and N-terminal domains
• in addition, **Scc1** binds **Scc3**

**Cohesion x evolution**

• stable sister-chromatid cohesion might be unique to eukaryotic cells
• bacterial sister origins move to opposite poles of the cell soon **after initiation** of DNA replication
• anaphase in bacteria commences long **before** chromosome replication is complete
Chromosome separation

separin

proteolysis
Lagging chromosomes emit a signal that induces the inactivation of APCcdc20 by a complex containing Mad1p, Mad2p and Mad3p.
S. cerevisiae kinetochore structure

• active centromeres recruit a high concentration of cohesin
• activation of APC leads to securin/Pds1 degradation through the ubiquitin (ub) pathway
• this liberates the separin/Esp1-dependent protease resulting in double cleavage of the Scc1/Mcd1 cohesin
• release of sister chromatid cohesion in anaphase
Centromere and associated proteins in *S. pombe*.

- 40–120 kb heterochromatic centromeres
- composed of distinct domains with different qualities of transcriptional silencing, and associated with specific proteins.

Schematic representation of metazoan kinetochore architecture and location of associated proteins.
END
Aurora B

- merotelic attachment
- monotelic attachment
- syntelic attachment

- Aurora B

- kinesin
- Microtubule emanating from opposite pole
- Spindle checkpoint activation state

- amphitelic attachment

- early prophase
- late prophase
- prometaphase
- metaphase
- anaphase
- telophase

- DNA
- aurora B

- Centromeres
- spindle
- midbody
TELOMERIC REPEATS

• in almost all eukaryotes capped with tandem copies of a simple DNA sequence – the telomeric repeat

• predominantly double stranded, with a 3' single-stranded overhang

• in mammalian cells, the length of the doublestranded repeat—(TTAGGG/CCCTAA)n—ranges from a few to tens of kbp

• the length of the singlestranded overhang—(TTAGGG)n—is a few hundred nucleotides at most
Function of telomeric repeats

- telomeric DNA and its associated proteins prevent illegitimate recombination and repair
- they establish a stable linear chromosome end
- they participate in chromosome positioning and segregation functions.
The maintenance of telomeric repeats cycles of cell division requires telomerase, a telomere-specific ribonucleoprotein (RNP) reverse transcriptase (reviewed in [2]). Telomerase copies a template sequence carried within its integral RNA and adds single-stranded telomeric repeats to the chromosome 3 ends. Single-strand synthesis by telomerase and accompanying complementary-strand synthesis can balance the telomere erosion inherent in incomplete end-
Cohesins and centromere

POLYTENE CHROMOSOMES
*Drosophila melanogaster*